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Dated 3 June 1999

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09JUN98 E366536-6 D02847
P01/7700 25.00 - 9812316.9

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

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Cardiff Road
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1. Your reference — HL59644/000/LCS

2. Patent application number
(The Patent Office will fill in this part) — **9812316.9**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

University of Bristol
Senate House
Tyndall Avenue
Bristol
BS8 1TH

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention
CANCER VACCINES

5. Full name of your agent *(if you have one)* — Haseltine Lake & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Imperial House
15–19 Kingsway
London WC2B 6UD

Patents ADP number *(if you know it)* — 34001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to a grant of patent required in support of this request? *(Answer "Yes" if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
Description	5
Claim(s)	1
Abstract	0
Drawing(s)	0

10. If you are also filing any of the following, state how many against each item.

Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to a grant of patent *(Patents Form 7/77*	0
Request for preliminary examination and search *Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(please specify*	0

11. I/We request the grant of a patent on the basis of this applicatio

Signature: Haseltine Lake & Co. (DAW)

Date: 5th June 1998

12. Name and daytime telephone number of person to contact in the United Kingdom: Dr. L.C. Sealy [0117] 9260197

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Notes

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CANCER VACCINES

The present invention relates to vaccines for a variety of diseases, particularly cancers, in which Epstein-Barr virus (EBV) has, or is believed to have, a causative role, and therapies for treatment of such diseases.

BACKGROUND

EBV is one of the eight known human herpes viruses. Infection usually occurs in early childhood; however, clinical symptoms are usually weak or undetectable at this stage. Primary infection with EBV later in life is associated with infectious mononucleosis (IM), which is the second most frequent disease in adolescence in the US. EBV also has oncogenic potential. There is a strong link between EBV and endemic Burkitt's lymphoma (BL) and undifferentiated nasopharyngeal carcinoma (NPC). Also, a large proportion of lymphomas that occur in immuno-compromised patients are caused by EBV, and an association has been shown to exist between certain Hodgkin's lymphomas and EBV.

Latently EBV-infected cells express a small number of so-called "latent" proteins. These include six nuclear proteins (EBNAs 1, 2, 3A, 3B, 3C and -LP), three integral membrane proteins (LMP-1, 2A and 2B) and two non-polyadenylated virus derived RNAs (EBERs) with a role in RNA splicing.

EBV latent membrane protein 1 (LMP-1) is present in the plasma membrane of infected cells. It is also expressed in nasopharyngeal carcinomas (NPCs) and EBV-positive Hodgkin's lymphomas (HD) which indicates a role for LMP-1 in the development of these tumours. The LMP-1 gene can alter the phenotype of uninfected cells causing the upregulation of cell surface activation markers, promoting cell proliferation. LMP-1 can also alter signalling pathways and has anti-

apoptotic effects. An cellular immune response directed against this viral antigen has not been demonstrated with any degree of certainty in either healthy carriers or tumour patients.

Many animal viruses have evolved mechanisms to avoid detection by the host immune system. Commonly, these mechanisms involve interference with the TAP-associated peptide translocation system. It is thought that EBV has also evolved similar mechanisms to avoid immune system detection, thus allowing its persistence in the host. This explains why certain cellular immune responses are not detectable to the EBV latent protein EBNA1 and could explain the apparent absence of such responses against LMP1.

The heat-labile enterotoxin of *E. coli* (Etx) and its homologue cholera toxin (Ctx) are composed of a single A subunit and five identical B subunits. The A subunit is toxic, exhibiting adenosine diphosphate (ADP)-ribosyl transferase activity. The B subunits (EtxB and CtxB) are non-toxic lectins that bind the cell surface glycolipid, ganglioside GM1, with high affinity and specificity, and cross-link it.

Binding of GM1 by EtxB has been shown to induce differential affects on lymphocyte populations, including a specific depletion of $CD8^+$ T-cells and an associated activation of B cells. WO 97/02045 (Williams *et al.*) discloses the use of agents such as EtxB for the treatment of autoimmune diseases, leukaemia's of T-cell origin, and for use in the prevention of transplantation rejection and graft versus host disease (GVHD).

THE INVENTION

According to a first aspect of the invention there is provided a vaccine composition which comprises:

a) EtxB, CtxB, or an agent other than EtxB or CtxB which has GM1-binding activity; and

b) an EBV antigen

for use in the treatment and/or prevention of EBV-associated diseases.

According to a second aspect of the invention there is provided a therapeutic composition which comprises:

EtxB, CtxB or an agent other than EtxB or CtxB which has GM1-binding activity

for use in the treatment of EBV-associated diseases.

Based on experiments carried out by the inventor which show that EtxB cocaps with LMP1, and that EtxB promotes fragmentation of LMP-1, it is theorised that EtxB (and other agents like CtxB having GM1 binding activity) will be useful to stimulate anti-EBV immune responses. This activity has applications in vaccines to prevent EBV associated diseases, and in therapeutic treatments to treat such diseases once they have developed.

Without wishing to be bound by theory, it is believed that when EtxB cocaps with LMP-1 the antigen is processed by a different intracellular route, which enables the antigen to by-pass the normal processing route which is blocked by the virus. The antigen is thus presented efficiently on the cell surface. The action of EtxB may also cause different epitopes of the antigen to be presented at the cell surface, from those which are presented if the antigen were processed by the conventional route.

The vaccine of the invention may be used to prevent infection by EBV, or development of EBV-associated diseases in EBV-infected individuals. The vaccine may also comprise a separate adjuvant, or the agent (such as EtxB or CtxB) can act as an adjuvant in its own right.

The agents specified in the second aspect of the

present invention may be used alone (i.e. without antigen) in the treatment of a EBV-associated disease which has already developed in a subject.

The terms "CtxB" and "EtxB" as used herein include natural and recombinant forms of the molecule. The recombinant form is particularly preferred, because it is in a pure form without any associated toxic A subunit. The terms also include mutant molecules and other synthetic molecules (containing parts of CtxB or EtxB) which retain the desired properties of CtxB or EtxB. Agents other than EtxB and CtxB which retain GM1-binding activity include antibodies which bind GM1. Humanised monoclonal antibodies are especially preferred. In all aspects of the invention, the agent having GM1-binding activity may also be capable of cross-linking GM1 receptors. EtxB is one such agent which is capable of cross-linking GM1 receptors by virtue of its pentameric form.

The preferred agent for use in the invention is EtxB.

The EBV antigen is an antigen derivable from EBV itself or an antigen which is caused to be expressed by an EBV-infected host cell by the action of EBV. Preferably the antigen is an EBV latent membrane protein. Particularly preferred are the antigens LMP-1, LMP-2A, LMP-2B, and EBNA-1 as well as antigenic fragments thereof. The antigen may be isolated directly from EBV infected cells, or be made by synthetic or recombinant means.

The term "antigen" includes proteins, glycoproteins, carbohydrates and nucleic acids. For protein antigens, the antigen may be administered in the vaccine in the form of whole protein, fragments of the protein which retain antigenic activity, or antigenic determinants.

The present invention is particularly suited for

the treatment and/or prevention of the following diseases: infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinomas, and Hodgkin's lymphomas. It is believed that the invention will be particularly suited to the treatment and/or prevention of nasopharyngeal carcinomas and Hodgkin's lymphomas.

The vaccine or the therapeutic composition according to the first and second aspects of the invention may be used to prevent development of, or treat, an EBV-associated disease in a mammalian subject, by administration of an immunologically effective amount to the subject.

The mammalian subject may be, for example, a healthy EBV-infected or uninfected individual, an immunodeficient individual, or an individual with an EBV-associated disease.

The vaccine may be administered by any suitable route. The agent and the antigen may be co-administered to the mammalian subject or administered separately. The agent and the antigen may be separate or linked, for example covalently or genetically linked, to form a single effective agent.

CLAIMS

1. A vaccine composition which comprises:

a) EtxB, CtxB, or an agent other than EtxB or CtxB which has GM1-binding activity; and

b) an EBV antigen

for use in the treatment and/or prevention of EBV-associated diseases.

2. A vaccine composition according to claim 1, which comprises EtxB.

3. A vaccine composition according to claim 1 or 2, wherein the EBV antigen is LMP-1.

4. A vaccine composition according to claim 1, 2 or 3, wherein the EBV-associated disease is a nasopharyngeal carcinoma or a Hodgkin's lymphoma.

5. A therapeutic composition which comprises:

EtxB, CtxB or an agent other than EtxB or CtxB which has GM1-binding activity

for use in the treatment of EBV-associated diseases.

6. A therapeutic composition according to claim 5, which comprises EtxB.

7. A therapeutic composition according to claim 5 or 6, wherein the EBV-associated disease is a nasopharyngeal carcinoma or a Hodgkin's lymphoma.